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Pipeline materials modify the effectiveness of disinfectants in drinking water distribution systems

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Abstract

We studied how pipe material can modify the effectiveness of UV- and chlorine disinfection in drinking water and biofilms. This study was done with two pipe materials: copper and composite plastic (polyethylene, PE) in a pilot scale water distribution network. UV-disinfection decreased viable bacterial numbers in the pilot waterworks and outlet water of pipes on average by 79%, but in biofilms its disinfecting effect was minor. Chlorine decreased effectively the microbial numbers in water and biofilms of PE pipes. In outlet water from copper pipes, the effect of chlorination was weaker; microbial numbers increased back to the level before chlorination within a few days. In the biofilms present in the copper pipes, chlorine decreased microbial numbers only in front of the pipeline. One reason for weaker efficiency of chlorine in copper pipes was that its concentration declined more rapidly in the copper pipes than in the PE pipes. These results means that copper pipes may require a higher chlorine dosage than plastic pipes to achieve effective disinfection of the pipes.

Keywords: Biofilm; Microbes; Copper; Plastic; Chlorine; UV-disinfection

1. Introduction

Most microbes in drinking water distribution system are present in biofilms inhabiting on the inner surfaces of pipelines (Laurent et al., 1993; Zacheus et al., 2001). Usually microbes in biofilms are more resistent against chemical disinfection than planktonic bacteria in water (LeChevallier, 1990). There are several reasons for invulnerability of bacteria in biofilms; including slow growth, physiologic heterogeneity of bacteria and sticky matrix containing DNA, other polymers and exopolysaccharides (Jefferson, 2004).

Traditionally drinking waters are disinfected by chlorine, chlorine dioxide or chloramine. However, nowadays some cities in Europe no longer utilize any disinfection chemicals to their distributed water (Uhl et al., 2001; Van der Kooij et al., 1998) or only UV-radiation is used. This is possible in a high-quality distribution system where the concentration of nutrients is low, the water temperature is low and where the

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retention time of water in distribution network is short (Uhl et al., 2001; Van der Kooij et al., 1998). In Finland, UV-disinfection of drinking water is becoming more common. One drawback to UV-disinfection is that UV-radiation has no residual effect within the distribution network. In large waterworks, UV-irradiation is usually followed by low dosing of chlorine, but in small ground waterworks, UV-irradiation is generally the only disinfection method.

Often the most problematic part of the distribution system is the household plumbing where there are increases in the temperature and concentrations of metals like copper and iron and the content of chlorine decreases (Zacheus and Martikainen, 1997). This can lead to an increase in microbial numbers in the water distributed throughout the buildings (Zacheus and Martikainen, 1995). There are studies showing that *Legionella* and *Mycobacteria* can grow in drinking water, this being a special problem in hot water distribution systems of hospitals, which may pose a health risk to the patients (Dailloux et al., 1999; Steinert et al., 2002; Norton et al., 2004). Usually household plumbing is constructed of plastic or copper, in some certain cases of stainless steel. There are previous studies showing that the characteristics of the pipe material can influence the formation of biofilms and the survival of pathogens in drinking water (Schwartz et al., 2003; Niquette et al., 2000; Norton et al., 2004).

In Kuopio, Finland, we have recently set up a pilot scale drinking water distribution system with commonly used materials in households: copper and plastic (polyethylene, PE). This pilot distribution system is built inside the building and is simulating the cold water plumbing systems present in domestic households. We have shown that the formation rate and the microbial community structure of biofilms were different in PE and copper pipes (Lehtola et al., 2004). In this study, the distribution network was connected to pilot scale waterworks and we tested whether the UV-disinfection and chlorination could change the drinking water chemical quality and microbial growth in the water and in the biofilms growing in pipelines with these different materials.

2. Materials and methods

2.1. Distribution networks

The pilot scale distribution networks consisted of two parallel 100 m loops of 50 mm (inner diameter, ID) PE pipes. One of those loops was connected to two parallel 10 mm (ID) copper- and other one to 12 mm (ID) composite (polyethylene–aluminum–polyethylene) plastic tubings (PE) (Fig. 1). The pilot distribution network of 10 and 12 mm pipes was run with tap water of Kuopio city for 1 year before the disinfection experiments (Lehtola et al., 2004). The larger 50 mm pipe was connected to the system just before these experiments, the pipe was flushed with the pilot water for approximately 30 min with a flow rate of 3–4 l/min (0.025–0.034 m/s) before use. Larger pipes were connected to the system to simulate the consumption of chlorine and increase of bacterial numbers in water distribution networks.

Water flow in PE pipes was on average 121 ml/min ($Re = 186$, laminar flow) and in copper pipes 118 ml/min ($Re = 215$, laminar flow) and the water pressure was 2.5 bar. Water flow was constant during the experiments. The water retention times in 50 mm pipes, 10 mm

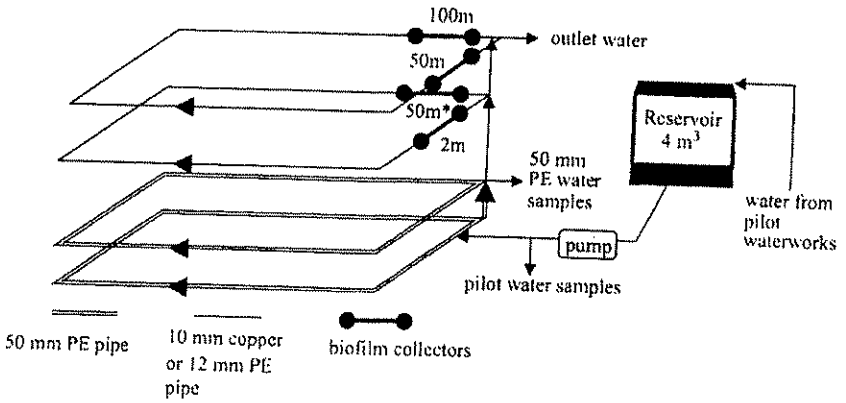

Fig. 1. Schematic representation of one pipeline in the pilot distribution network. There were two parallel 100 m loops of 50 mm PE pipelines, from which one was connected to two parallel 100 m loops of 10 mm copper pipelines and one to two parallel 100 loops of 12 mm PE pipelines. Codes of the biofilm collectors represent the distance from the beginning of the pipe, new 50 m biofilm collector is marked by asterisk.

copper pipes and 12 mm PE pipes were 27 h, 65 min and 94 min, respectively. The drinking water used in the disinfection experiments was produced in pilot scale waterworks, where lake water was chemically coagulated with ferric sulphate, flotated, rapid sand filtrated and finally water hardness, alkalinity and pH were adjusted with lime and carbon dioxide. The waterworks produced water at a flow rate of 1.2 m^3/h. The purified water was collected into a 4 m^3 stainless-steel reservoir pool before pumping to the pilot distribution network. Overflow was drained to the sewer. Water samples were taken after 50 mm pipes (PE50), after two parallel PE pipes (PE) and two parallel copper pipes (Cu) (Fig. 1). Pilot water samples were taken after the water reservoir (Fig. 1).

Disinfection experiments were conducted in three 3 week periods. During the first period there was no disinfection of the water, in the second period (days 18–39) the drinking water was disinfected with UV-radiation (70 mW s/cm^2) produced by a low-pressure UV-lamp and in the last period (days 39–61) the drinking water was disinfected with both UV-radiation and chlorine (NaOCl). Chlorine was added at a final concentration of approximately 2 mg/l (Table 1). Water samples were taken three times per week.

2.2. Water analyses

The drinking water quality characteristics are presented in Table 1. Analyses were undertaken in a certified laboratory using standard methods.

Total non-purgeable organic carbon (TOC) was analysed by a high-temperature combustion method with a Shimadzu 5000 TOC analyser (Kyoto, Japan). Assimilable organic carbon (AOC) was analysed by a modification (Miettinen et al., 1999) of the Van der Kooij et al.'s method (1982). The modification included addition of inorganic nutrients such that AOC was measured as $AOC_{potential}$ (Miettinen et al., 1999). The growth of *Pseudomonas fluorescens* P17 (ATCC 49642) and *Spirillum* sp. strain NOX (ATCC 49643) in water samples was calculated to correspond to acetate equivalents. In water samples containing chlorine, residual chlorine was removed by the addition of 50 µl 0.02 M (for 100 ml) sodium thiosulphate.

Total phosphorus (total P) was analysed using the ascorbic acid method according to the Finnish standards (SFS-EN 1189, 1997). Absorbance was measured spectrophotometrically (Shimadzu UV-1601, Australia) at 880 nm wavelength using a 5 cm light path. Microbially available phosphorus (MAP) was analysed with a bioassay where the maximum growth of *P. fluorescens* P17 (ATCC 49642) in pasteurized water samples is related to the phosphorus concentration (Lehtola et al., 1999). Inorganic nutrients (except phosphorus) and sodium acetate were added to the water to ensure that the growth of test bacteria was limited solely by phosphorus. The maximum microbial cell production (cfu/ml) was converted to the phosphorus concentration using the empirical yield factor of 3.83×10^8 cfu/µg P (Lehtola et al., 1999).

Heterotrophic bacteria were analysed with a spread plating method on R2A-agar (Difco) (Reasoner and Geldreich, 1985). R2A-agar plates were incubated for 7 days at 22 °C before colony counting (cfu).

Virus-like particles (VLP) and bacteria for direct counts were stained with SYBR green I (Sigma, St. Louis, USA) nucleic acid stain (Noble, 2001; Rinta-Kanto et al., 2004). Samples were preserved by adding 37% formaldehyde to obtain a final concentration of 2%. The water sample of 800 µl was filtered through a 0.02 µm pore size aluminium oxide filter (Anodisc 25,

Table 1
Water quality characteristics of Kuopio tap water and water produced in pilot waterworks

		Kuopio tap water $n = 6$	Pilot $n = 13$	Pilot + UV $n = 12$	Pilot + UV + Cl $n = 16$
Temperature	°C	11.9 ± 0.9	8.7 ± 1.4	9.1 ± 2.9	12.2 ± 0.6
pH		8.0 ± 0.1	7.6 ± 0.1	7.7 ± 0	7.5 ± 0.1
Conductivity	µS/m	166 ± 18	187 ± 9	187 ± 3	191 ± 5
Alkalinity	mmol/l	0.97 ± 0.1	0.88 ± 0.06	0.86 ± 0.02	0.75 ± 0.04
Hardness	mmol/l	0.57 ± 0.04	0.49 ± 0.16	0.40 ± 0.04	0.36 ± 0.05
Iron	mg/l	0.05 ± 0.02	0.08 ± 0.03	0.06 ± 0.01	0.06 ± 0.02
Sulphate	mg/l	30.2 ± 1.3	36.5 ± 3.5 (2)	n.a.	n.a.
Chloride Cl^-	mg/l	7.3 ± 0.9	3.2 ± 0.1	3.2 ± 0.1	6.2 ± 0.3
Chlorine	mg/l	0.06 ± 0.04 (18)	—	—	1.87 ± 0.23
TOC	mg/l	3.2 ± 0.5	3.1 ± 0.0	3.1 ± 0.0	2.9 ± 0.1
AOC	µg/l	41 ± 20 (11)	46 ± 14 (3)	27 ± 20 (3)	41 ± 10 (3)
MAP	µg/l	0.20 ± 0.17 (12)	0.09 ± 0.06 (3)	0.11 ± 0.08 (3)	0.06 ± 0.02 (3)

In chlorine, sulphate, AOC and MAP the number of analyses is in the parenthesis. TOC, total organic carbon; AOC, assimilable organic carbon; MAP, microbially available phosphorus.

Whatman Ltd., Maidstone, England). After collecting of bacteria and VLPs on the filters, they were stained with SYBR green I (Sigma) nucleic acid stain (final dilution 0.25%) and enumerated using ×1000 magnification under blue excitation using an Olympus BX 51TF epifluorescence microscope (Olympus Co. Ltd., Japan) equipped with an ocular grid. Bacteria and VLPs were distinguished based on their dimensions and or their relative brightness (Noble, 2001).

The growth potential of native microbial populations (HGR) in water was analysed by incubating the samples at 15 °C in the dark. Bacterial growth was followed for 3 weeks by spread plating every second or third day on R2A-agar plates (Difco) (Reasoner and Geldreich, 1985). R2A-agar plates were incubated for 7 days at 22 °C before cfu's were counted. The maximum microbial numbers obtained during the water incubation are reported.

Soluble copper in water was analysed using the bicinchoninate method with a HACH DR/2010 spectrophotometer (Loveland, Colo. USA) and Cuver® 1 copper reagent (HACH Permachem reagents, USA). Free chlorine residual was analysed with a Palinest Micro 1000 chlorometer (Palintest Ltd., England).

2.3. Biofilms

Biofilm collectors were installed as components of the pilot distribution system. The collectors consisted of 15 cm (copper) and 20 cm (PE) pieces of pipes, which were connected in line and installed as a part of the system by ball valves at the ends of the biofilm collector pipes. Biofilm collectors were installed at 2, 50 and 100 m distances from the beginning of the pipeline. Some of the biofilm collectors (PE 2 m old, PE 50 m old, Cu 2 m old and Cu 50 m old, m means the distance as meters of the collector from the beginning of the pipeline) were installed to the distribution network about 6 months before connecting the pilot waterworks to the network, i.e. these old collectors were fed by Kuopio tap water before connecting to the pilot waterworks. This arrangement enabled us to study the effect of water quality change on the old biofilms. New collectors were installed just before connecting the pilot to the network, these collectors were disinfected with chlorine before use (12 mg/l for 2 h).

During sampling, the biofilm collector pipes were closed via ball valves before disconnecting and removed with the water inside the tube. In laboratory, ball valves were removed and the pipes were closed with ethanol swabbed parafilm (American National Can, USA). The biofilms inside the collector pipes were removed by shaking with sterile 2 mm glass beads and rinsing with 5 ml sterile water (Zacheus et al., 2000). The results are given as an average of two parallel pipelines.

For total bacteria and VLP analyses biofilm extraction of 100 µl was diluted with sterile water to 800 µl and analysed as described above. HPC in the biofilm extraction was analysed as described above for water. All biofilm results were normalized to the amount of surface area (cm^2).

3. Statistical methods

The statistical differences for the parameters were analysed with oneway analysis of variance calculated with Microsoft Excel 2000 program.

4. Results

4.1. Pilot water without disinfection

There were some differences in drinking water quality between Kuopio tap water and water produced in pilot waterworks (Table 1). In the water produced in pilot waterworks pH ($p<0.001$) and alkalinity ($p<0.01$) were lower and conductivity ($p<0.01$) higher than in Kuopio tap water (Table 1). The concentrations of AOC were similar in these waters. The concentration of MAP was lower in water produced in the pilot waterworks, but due the high variation in results the difference was not statistically significant (Table 1).

The copper concentration in outlet water of copper pipes increased from 0.23 ± 0.05 to 0.53 ± 0.09 mg/l ($p<0.001$) after the pilot waterworks was connected to the distribution network. It was not possible to analyse the concentrations of AOC and MAP in the outlet water of copper pipes, because the water was toxic for the *P. fluorescens* strain used in the AOC and MAP bioassays. Both total phosphorus and MAP concentrations clearly increased in 50 mm pipeline ($p<0.001$), and were higher also in outlet waters of PE pipes ($p<0.01$ for MAP and $p<0.001$ for total phosphorus) (Fig. 2). MAP concentration started to decrease with time (Fig. 2), but total phosphorus remained high (17 ± 2 µg/l P) for the first 4 weeks. At the end of all experiments, total phosphorus in outlet waters was on average 11 ± 2 µg/l P, but MAP had decreased to below 1 µg/l P (Fig. 2). The AOC concentration decreased strongly ($p<0.001$) in the distribution networks (Fig. 3). Changes in nutrient concentrations were related to the microbial regrowth potential in water (Fig. 4). Microbial numbers in outlet water increased ($p<0.001$) after connecting the pilot waterworks to the networks (Table 2). The number of HPC in pilot water did not differ from Kuopio tap water (Table 2).

After the first 3 weeks period, the change in water source from Kuopio tap water to water produced in pilot waterworks did not result in significant changes in

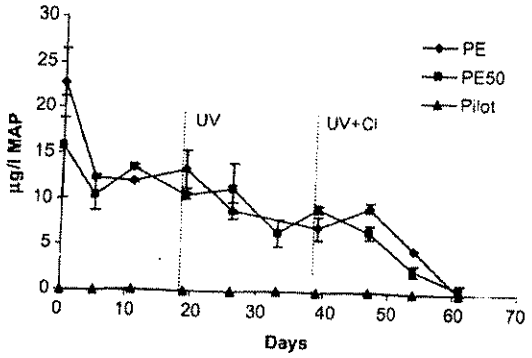

Fig. 2. Concentration of MAP in water from pilot waterworks and outlet waters of PE pipes. PE: outlet waters from 12 mm PE pipes (mean of two parallel pipes ± range), PE50: outlet waters from 50 mm pipes (mean of two parallel pipes ± range), Pilot: inlet water produced in pilot waterworks. Dashed line shows the time when UV-disinfection or UV + chlorination were initiated.

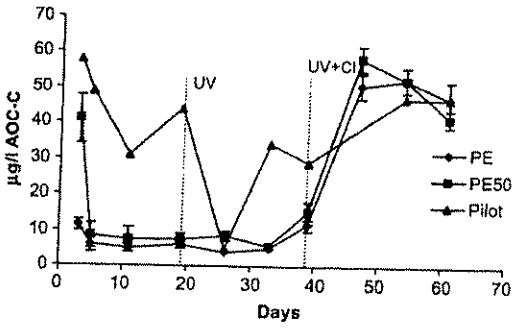

Fig. 3. Concentration of AOC in water from pilot waterworks and outlet waters of PE pipes. PE: outlet waters from 12 mm PE pipes (mean of two parallel pipes ± range), PE50: outlet waters from 50 mm pipes (mean of two parallel pipes ± range), Pilot: inlet water produced in pilot waterworks. Dashed line shows the time when UV-disinfection or UV + chlorination were initiated.

the number of bacteria in biofilms (Figs. 5 and 6). In old biofilms of the copper pipes, the number of VLPs increased ($p = 0.05$) after pilot waterworks was connected to the distribution network (Fig. 7). The number of VLPs was lower ($p < 0.001$) in copper pipes than the corresponding value in PE pipes (Fig. 7). When compared old biofilm collectors, there were no statistically significant differences in the number of HPC or total number of bacteria in biofilms of copper pipes or PE pipes (Fig. 6). In the new biofilm collectors, the total number of bacteria was higher ($p < 0.05$) in PE pipes than in the copper pipes (Figs. 5 and 6).

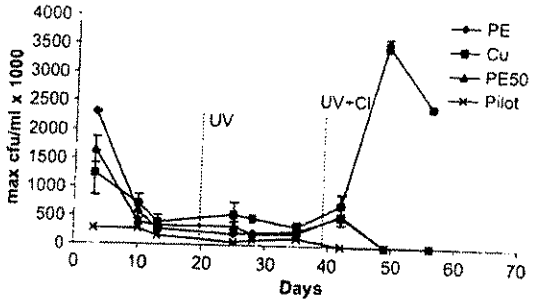

Fig. 4. Maximum growth potential of microbes in water from pilot waterworks and outlet waters of PE and copper pipes. PE: outlet waters from 12 mm PE pipes (mean of two parallel pipes ± range), Cu: outlet waters from copper pipes (mean of two parallel pipes ± range), PE50: outlet waters from 50 mm pipes (mean of two parallel pipes ± range), Pilot: inlet water produced in pilot waterworks. Dashed line shows the time when UV-disinfection or UV + chlorination were initiated.

4.2. UV-disinfection

UV-disinfection had no significant effects on MAP or AOC concentrations in pilot water (Figs. 2 and 3). The concentration of copper in outlet water of copper pipes increased to an average of 0.69 ± 0.08 ($p < 0.001$) mg/l after UV-disinfection was initiated.

UV-disinfection decreased the number of HPC in the pilot waterworks on average 72% ($p < 0.001$). In the distribution network the decrease in 50 mm pipes was on average 82% ($p < 0.001$), in the outlet water of PE pipes on average 84% ($p < 0.001$) and in the outlet water of copper pipes on average 76% ($p < 0.001$) (Table 2). UV-disinfection had only a minor effect on the numbers of total bacteria and VLPs (Table 2).

UV-disinfection had no significant effects on microbial numbers in the biofilms of PE or copper pipes (Figs. 5–7). However, the number of VLPs increased ($p < 0.05$) in biofilms growing in copper pipes (Fig. 7).

4.3. UV-disinfection+chlorination

Chlorination had no significant effect on MAP concentration in the pilot waterworks (Table 1). Concentration of AOC increased in pilot waterworks to an average of 41 µg/l C (Table 1, Fig. 3) and it continued increasing in the networks up to 50–60 µg/l C (Fig. 3). The increase of AOC was not statistically significant in pilot waterworks, but in networks the increase was significant ($p < 0.001$). The concentration of copper in outlet water of copper pipes increased to an average of 0.78 ± 0.08 ($p < 0.001$) mg/l.

The concentration of free chlorine in pilot water was on average 1.9 ± 0.2 mg/l, after 50 mm pipes it was 0.86 ± 0.19 mg/l ($p < 0.001$), after PE pipes

Table 2
The effects of different disinfection options on water microbiological quality in pilot scale distribution network

	HPC (×1000 cfu/ml)				Total bacteria × 1000/ml				VLP × 1000/ml			
	Kuopio tap water	Pilot	Pilot + UV	Pilot + UV + Cl	Kuopio tap water	Pilot	Pilot + UV	Pilot + UV + Cl	Kuopio tap water	Pilot	Pilot + UV	Pilot + UV + Cl
Inlet water												
Avg.	1.9	2.2	0.6	0.01	160	150	195	50	3830	4002	4431	400
sd	±1.4	±0.5	±0.3	±0.02	±35	±58	±31	±29	±615	±866	±474	±156
n	9	7	14	10	5	3	3	2	6	3	3	2
PE 50 mm												
Avg.		208	37	0.01		468	360	212		4644	5020	2362
sd		±95	±7	±0.02		±231	±57	±167		±761	±646	±2515
n		12	14	16		6	6	4		6	6	4
PE												
Avg.	5.6	240	38	0.01	211	458	387	139	4129	3984	4045	899
sd	±1.2	±91	±16	±0.01	±29	±211	±55	±133	±688	±1295	±893	±1141
n	16	12	14	16	12	8	6	6	14	8	6	6
Cu												
Avg.	7.9	143	35	19	188	365	242	176	2756	139	86	147
sd	±5.1	±47	±11	±22	±32	±175	±107	±79	±461	±125	±46	±237
n	16	12	14	16	12	8	6	6	14	8	6	6

HPC: heterotrophic plate counts, VLP: virus-like particles.

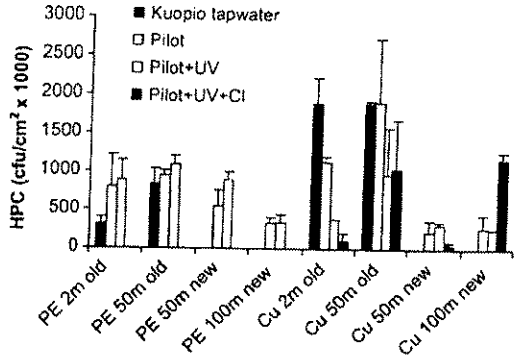

Fig. 5. Number of HPC in biofilms growing on copper and plastic pipes (mean of two parallel pipes ± range) in different parts of the pipeline (meters from the beginning). Old biofilm collectors were installed 6 months before connecting of the pilot waterworks to the network, new collectors were installed just before connecting to the pilot waterworks.

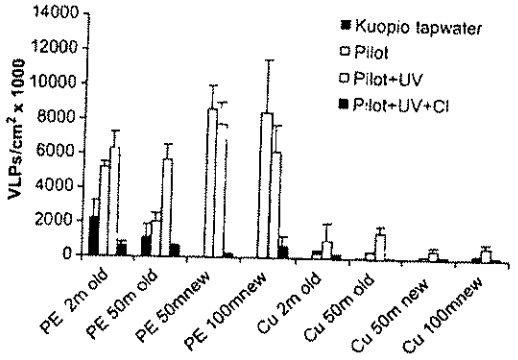

Fig. 7. Number of virus-like particles (VLPs) in biofilms growing on copper and plastic pipes (mean of two parallel pipes ± range) in different parts of the pipeline (meters from the beginning). Old biofilm collectors were installed 6 months before connecting the pilot waterworks to the network, new collectors were installed just before connecting to the pilot waterworks.

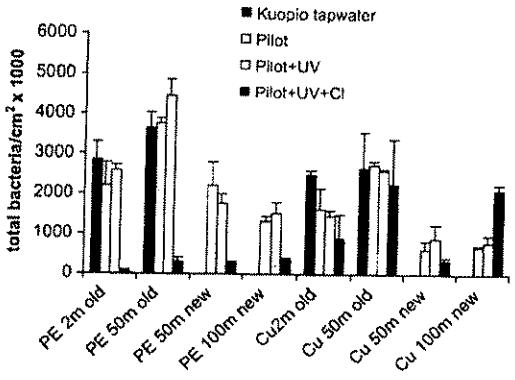

Fig. 6. Number of total bacteria in biofilms growing on copper and plastic pipes (mean of two parallel pipes ± range) in different parts of the pipeline (meters from the beginning). Old biofilm collectors were installed 6 months before connecting the pilot waterworks to the network, new collectors were installed just before connecting to the pilot waterworks.

0.59 ± 0.15 mg/l ($p<0.001$ if compared to 50 mm pipes) and after the copper pipes 0.13 ± 0.05 mg/l ($p<0.001$ if compared to 50 mm pipes). Concentration of chlorine in water was significantly lower ($p<0.001$) after copper pipes than it was after PE pipes. Chlorine eliminated effectively culturable bacteria in pilot ($p<0.001$) waterworks and outlet water of PE pipes ($p<0.001$) (Table 2). Also, in PE pipes total number of bacteria ($p<0.01$) and number of VLPs ($p<0.001$) in water decreased after chlorination. HPC in outlet water of the copper pipes decreased strongly ($p<0.001$) for 1 week, but after that time the HPC recovered back to the level before the start of chlorination. Also, HGR in outlet water of copper pipes increased strongly ($p<0.01$) (Fig. 4). HGR could not be determined from outlet water of PE pipes, because the water contained no culturable bacteria.

Chlorination effectively eliminated culturable bacteria ($p<0.001$), total number of bacteria ($p<0.01$) and VLPs ($p<0.001$) in biofilms of the PE pipes (Fig. 5). In copper pipes, chlorine had a lesser effect on biofilms. Chlorine decreased the number of culturable bacteria in front of the copper pipe (Cu 2 m) and in new biofilms in the middle part (Cu 50 m new) of the pipeline ($p<0.01$) (Fig. 5). In the old biofilms growing in the middle of the pipeline, chlorination did not have any effect and at the end of the pipeline, the microbial numbers in biofilms even demonstrated an increase ($p<0.01$ for HPC and $p<0.05$ for total number of bacteria) (Figs. 5 and 6). The number of VLPs in copper pipes decreased after chlorination ($p<0.01$) (Fig. 7).

5. Discussion

We have found earlier that PE pipes may release phosphorus into drinking water (Lehtola et al., 2004). Even though the 50 mm pipe was flushed before use in the present study, the contents of both total phosphorus and MAP increased strongly in water. As a result of the flow through the 50 mm PE pipes, the concentration of phosphorus in outlet water of pipes did decrease with time, which probably affected microbial growth. The concentration of phosphorus, especially MAP, was so high compared to AOC, that microbial growth in water

and biofilms was thought to be limited by the availability of organic carbon.

The increase in retention time and nutrient concentrations resulted in a major increase in microbial numbers in the outlet water of the distribution networks. The increase in microbial activity was associated with a decrease in concentration of AOC in the networks, supporting some previous results showing that microbial growth in networks can decrease the AOC content in water (LeChevallier et al., 1987; Van der Kooij, 1992; Miettinen et al., 1997). The increase in the concentration of copper may be a result of changes in corrosivity of water, or changes in microbial biomass and activity. In the pilot waterworks water alkalinity and pH were lower than in Kuopio tap water, i.e. pilot water was slightly more corrosive than Kuopio tap water. Critchley et al. (2001) have shown that biofilms may cause cuprosolvency. However, there are also opposite results showing a protective effect of biofilm on copper, and showing that cuprosolvency is dependent on the microbial population (Critchley et al., 2001; Critchley and Fallowfield, 2001). Disinfection of the water increased the copper concentration in the outlet water of copper pipes, which may be reflected in the protecting effects of biofilms on copper pipes. Because corrosion is based on oxidation/reduction reaction, the chlorine in water can also affect the copper corrosion (Rushing and Edwards, 2004). In our previous studies with Kuopio tap water, we detected positive correlation between HPC in inlet water and copper concentration in outlet water (Lehtola et al., 2004). These different results may be due to the different microbial populations in the inlet waters and to an increase in the water corrosivity when the water was disinfected.

In old biofilm collectors, the number of bacteria in biofilms did not significantly differ in copper and PE pipes, but in new collectors the number of bacteria was higher in PE pipes. This agrees with our previous results where we found that the formation of biofilm occurs more slowly in copper pipes than in PE pipes (Lehtola et al., 2004). The number of VLPs increased especially in the old biofilms of copper pipes after connecting the network to the pilot waterworks. This may be a consequence of the increase in bacterial activity in biofilms, triggered by the increase in availability of phosphorus (originating from the 50 mm PE pipe material). Bacteria are known to release larger numbers of viruses in conditions favouring fast growth and high productivity (Wommack and Colwell, 2000). The number of VLPs was lower in outlet water and biofilms of the copper pipes than in the PE pipes, which agrees with our previous results (Lehtola et al., 2004).

The effect of UV-disinfection on the number of bacteria in pilot water was lower than would have been anticipated from the literature, although the UV-dose (70 mW s/cm^2) used in these experiments was higher than normally used in waterworks (Harris et al., 1987; Parrotta and Bekdash, 1998). The reason for that might be related to the sampling procedure. Samples were taken after the water reservoir where the bacteria, which have survived UV-disinfection may grow in water or emerged from the biofilms. The water reservoir was not disinfected before the UV-disinfection was applied. Sampling after water reservoir was important, because then it was possible to analyse more accurately the effects of chlorination and pipeline material on the water quality. Typically, the number of bacteria increased and the concentration of free chlorine decreased in pipelines and water reservoir before the pilot distribution network (results not shown). However, the effect of UV-disinfection was seen also in bacterial numbers in the outlet waters. This means that even though UV-disinfection has no residual effects in networks, the decrease in bacterial concentration in waterworks results in a lower number of bacteria also in outlet water of the pipes. We have found earlier that the microbial community structure in water changes in this network (Lehtola et al., 2004). These findings suggest that bacteria in water originate both from biofilms and the inlet water, not solely from biofilms. UV-disinfection had no effect on bacterial numbers in the biofilms of PE- or copper pipes, but increased the number of VLPs in biofilms of copper pipes. It is not known why the VLPs in the biofilms present in copper pipes increased. In pilot waterworks, UV-disinfection had no effects on MAP, which confirms our previous findings with UV-doses below 200 mW s/cm^2 (Lehtola et al., 2003).

In the PE pipes, chlorine decreased effectively the number the HPC in water and biofilms. Also the total number of bacteria and VLPs decreased strongly after chlorination. These results suggest that even at chlorine concentrations of 0.86 mg/l, the concentration after 50 mm pipes, chlorine can destroy biofilms. In general higher concentrations of chlorine are needed to eliminate biofilm microbes (LeChevallier, 1990). Chlorine increased the content of AOC in water, in support of previous results (Van der Kooij, 1990; Miettinen et al., 1998; Lehtola et al., 2001a). In PE pipelines the content of AOC increased with retention time. There are two possible explanations for this phenomenon. First, there was no further microbial consumption of AOC, and second, chlorine could have been reacted with organic matter to generate a continuous supply of AOC. We have shown earlier that it can take 24 h or more to achieve the maximum increase in the content of AOC in drinking water after chlorine treatment (Lehtola et al., 2001a). In this study the age of water before entering the distribution network was no longer than few hours. Since the water still contained an excess of phosphorus, the increase in AOC clearly increased the growth potential of bacteria (HGR) in water, as seen in the

outlet waters from the copper pipes. Chlorination had no effects on MAP, as found earlier (Lehtola et al., 2001a). Release of MAP from humic substances requires stronger oxidants such as ozone (Lehtola et al., 2001b).

In the copper pipes, the disinfection efficiency of chlorine was lower than in the PE pipes. During the first days after chlorination, chlorine decreased effectively bacterial numbers in outlet water of the copper pipes, but subsequently the number of bacteria increased back to the level before chlorination. These bacteria were originating from the biofilms of the copper pipes, because after 50 mm PE pipes (inlet water of the copper pipes) there were no culturable bacteria in water. Biofilms and bacteria in the copper pipes might be better able to tolerate chlorine than the biofilms and bacteria in the PE pipes. In front of the PE and copper pipes, the content of chlorine was the same, but only in biofilms of copper pipes it was able to detect any culturable bacteria. There may be some genetic adaptation in the bacteria i.e. to produce oxidant-degrading and repair enzymes against oxidising agents (Cloete, 2003). Another reason may be that in the copper pipe, the chlorine concentration decreased faster than in the PE pipes. In outlet water of the copper pipes, the free chlorine concentration was only 0.13 mg/l, whereas after it passed through the PE pipes the water's chlorine level was still 0.59 mg/l. Chlorine can react with copper producing copper(I)chloride or copper(II)chloride (Cotton et al., 1987). CuCl is highly insoluble, but $CuCl_2$ is soluble. In aqueous solution Cu^{2+} is more prevailing state of copper (Cotton et al., 1987).

The decrease in chlorine concentration in copper pipes was seen on its effect on biofilms. In biofilms taken from the beginning of the copper pipe, chlorination lowered the numbers of bacteria, but at the end of the pipe the number of bacteria in biofilms increased strongly. This increase is probably due to the higher content of AOC in water, an important factor for microbial growth in biofilms in carbon limited water (Van der Kooij et al., 1995). In the mid-section of the pipe, the age of biofilm affected the impact of chlorine. Older biofilms were resistant to chlorine. The thicker old biofilms may provide better protection against chlorination and the microbial community living there would be better able to withstand the toxic effects of chlorine (Jefferson, 2004). There are known to be different microbial community structures in biofilms growing in PE pipes and in copper pipes (Lehtola et al., 2004).

In this work the chlorination was achieved with hypochlorite (NaOCl), which is more reactive than chloramine. LeChevallier et al. (1988) found that chloramine is a more effective disinfectant for biofilms than chlorine because it can penetrate deeper into the biofilms. It is possible that in copper pipes, chloramine would be more effective disinfectant than chlorine, a factor requiring further study in the future.

6. Conclusions

Our study showed that the effect of disinfection depends on the pipeline material. Even though UV-disinfection has no residuals in the distribution network, it decreased the microbial numbers in the outlet water in the network. Chlorination decreased effectively the microbial numbers in water and biofilms of PE pipes. In copper pipes, the effect of chlorine was less effective, probably due to the greater decrease in chlorine concentration occurring there and the better tolerance of bacteria against chlorine. The results show that it is important to analyse the chlorine dose in outlet waters of pipelines to ensure the adequate dosing of chlorine. Copper pipes may require a higher chlorine dosage than plastic pipes.

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